

## Production of Human Serum Proteins by Human Cancer Cell Lines Growing in Rats\* (33058)

ARTHUR G. LEVIN AND CHESTER M. SOUTHAM

*Sloan-Kettering Institute for Cancer Research, New York, New York 10021*

Certain human cell lines produce serum globulins in culture. These proteins were demonstrated by reaction with antisera to human serum protein components (1-6). Cell line J-111, which originated from the peripheral blood of a patient with monocytic leukemia (7) and line HEp 2, derived from epidermoid carcinoma of larynx (8,9) produce proteins which react with antisera to the  $\beta$ 1C component of human serum (5). These cell lines grow progressively when injected intravenously into newborn rats (10). The serum of such rats was investigated for the presence of human serum proteins. This report concerns only the reaction of such rat sera with the antiserum to human  $\beta$ 1C.

**Methods.** Newborn Wistar rats were injected intravenously with two million J-111 or HEp 2 cells from lines continuously maintained in culture in this laboratory for more than 10 years. The rats were sacrificed at 29-41 days of age when evidence of gross tumor growth was observed, and blood was obtained by heart puncture. Control rats of the same age, some times from littermates of the tumor bearing rats, were bled at the time.

Immunodiffusion studies were carried out in agar, "Immunoplates" Pattern B (distance between wells, 7 mm). The antiserum was goat antihuman  $\beta$ 1C/ $\beta$ 1A antiserum. The "Immunoplates" and the antiserum were purchased from Hyland Laboratories, Los Angeles, California. The antiserum was adsorbed by incubation with 10-20 mg of lyophilized normal rat serum per ml of antiserum. All wells receiving the rat sera were filled three times. Other wells were filled once. The

\* Study supported in part by grants from the National Cancer Institute USPHS (CA 08748) and the American Cancer Society (T 229). The authors wish to acknowledge the technical assistance of Adrienne Tanzi, Nella Shapiro, Debra Fox, and John Hlinka.

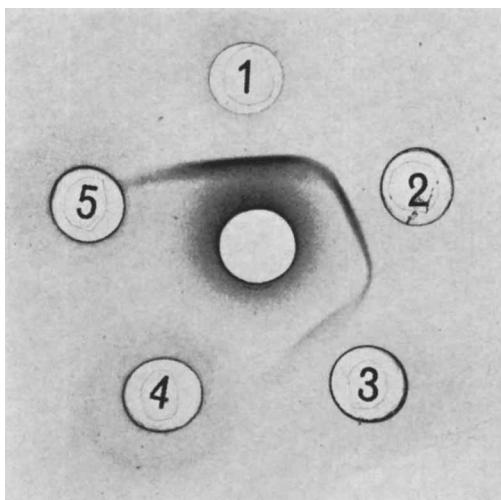


FIG. 1. Gel diffusion plate showing reaction between goat antihuman  $\beta$ 1C/ $\beta$ 1A (center well) and normal human serum (well 1) and serum from rats bearing J-111 (wells 2 and 3) but no reaction with serum from normal control rats (wells 4 and 5).

plates were incubated at room temperature for 18 hours.

**Results.** Ten serum samples from individual rats or groups of 2 or 3 rats bearing J-111 or HEp 2 were tested. Each reacted with antiserum to human  $\beta$ 1C/ $\beta$ 1A. Serum of control rats did not. Figure 1 shows an immunodiffusion plate in which the antihuman  $\beta$ 1C/ $\beta$ 1A (center well) reacted with the serum of two J-111 bearing rats (wells 2 and 3) and with human sera (well 1), but not with the serum of two control rats (wells 4 and 5). A continuous precipitin line is present between the center well and wells 1, 2, and 3 and is not seen between the center well and wells 4 and 5, indicating antigenic identity between the  $\beta$ 1C and/or  $\beta$ 1A human serum globulin and the reacting protein in the serum from the tumor bearing rats.

Similarly, Fig. 2 illustrates the precipitin reaction between the antihuman  $\beta$ 1C/ $\beta$ 1A (center well) and serum from a rat bearing HEp 2 (well 1), serum from a rat bearing

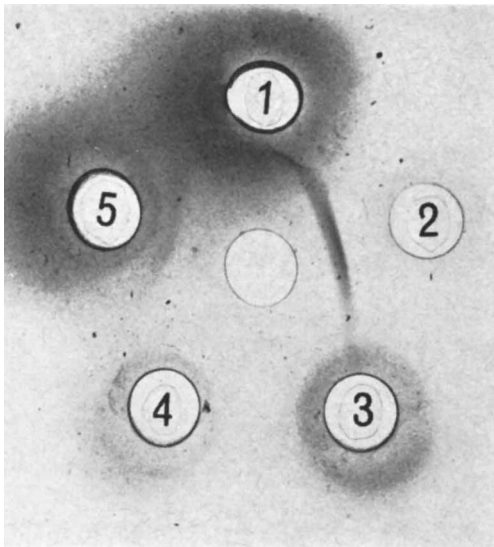


FIG. 2. Gel diffusion plate showing reaction between goat antihuman  $\beta 1C/\beta 1A$  (center well) and normal human serum (well 2) and serum from a rat bearing HEP 2 (well 1) and a rat bearing J-111 (well 3), but no reaction with serum from normal control rat (wells 4 and 5).

J-111 (well 5) and with normal human serum (well 2). The continuity of this precipitin line indicates antigenic identity amongst proteins in each of the three sera. There is no reaction with serum from two control rats (wells 3 and 4).

These results indicate that serum from rats bearing transplants of either of these two

human cell lines contains human  $\beta 1C$  and/or  $\beta 1A$  serum globulins, or proteins with identical antigenic determinants. It is presumed that these proteins were produced by the human cells growing in the rats.

*Summary.* Human serum globulin ( $\beta 1C$  and/or  $\beta 1A$ ) was demonstrated by the immunodiffusion technique in sera of rats bearing heterotransplants of human cancer cell lines HEP 2 or J-111, but not in serum of normal rats. Previous investigators have demonstrated that these cell lines produce  $\beta 1C$  globulin in tissue culture.

1. Fahey, J. L., Finegold, I., Manaker, R., and Rabson, A., *Science* **152**, 1257 (1966).
2. Tanigaki, N., Yagi, Y., Moore, G. E., and Pressman, D., *J. Immunol.* **97**, 634 (1966).
3. Trujillo, J. M., Butler, J. J., List-Young, B., Schullenberger, C. C. and Gott, C., *Nature* **209**, 310 (1966).
4. Finegold, I., Fahey, J. L., and Granger, H., *J. Immunol.* **99**, 839 (1967).
5. Stecher, V. J. and Thorbecke, G. J., *J. Immunol.* **99**, 660 (1967).
6. Wakefield, J. D., Thorbecke, G. J., Old, L. J., and Boyse, E. A., *J. Immunol.* in press.
7. Osgood, E. E. and Brooke, J. H., *Blood* **10**, 1010 (1955).
8. Moore, A. E., Sabachewsky, L., and Toolan, H. W., *Cancer Res.* **15**, 598 (1955).
9. Fjelde, A., *Cancer* **8**, 845 (1955).
10. Southam, C. N., Babcock, V. I., and De Masi, M., *Cancer Res.* **24**, 345 (1964).

Received Feb. 1, 1968. P.S.E.B.M., 1968, Vol. 128.

### Effect of Glucagon on Plasma Free Fatty Acids and Blood Sugar in Birds.\* (33059)

FRANCISCO GRANDE

*Jay Phillips Research Laboratory, Mount Sinai Hospital and Laboratory of Physiological Hygiene,  
University of Minnesota, Minneapolis, Minnesota 55455*

Glucagon stimulates *in vitro* the release of free fatty acids (FFA) by the adipose tissue of the domestic fowl (1) the sparrow, and the pigeon (2,3). Carlson *et al.* (1) have reported that, in contrast with mammalian adipose tissue, the adipose tissue of the domestic fowl does not respond with increased liberation of FFA or glycerol when treated *in vitro* with

epinephrine, norepinephrine, or other adipokinetic substances. Infusion of norepinephrine caused no change of FFA plasma concentration in the fowl (1).

\* Supported by a grant from the John A. Hartford Foundation, New York, and by the Mount Sinai Hospital Research Fund.